LABORATORY

Application Note

DNA Purification from PCR or Agarose Gels Using Pall Nucleic Acid Binding (NAB) Nanosep® Centrifugal Devices

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Introduction

The Pall Nanosep centrifugal device for nucleic acid binding (NAB) incorporates a dual layer silica-based quartz glass fiber media to allow for efficient binding of DNA and RNA, while providing smooth flow and rapid processing of samples. The NAB Nanosep device was originally shown to purify plasmid DNA, genomic DNA, and total RNA from a variety of starting materials. Here we show that the NAB Nanosep device can also be used to purify DNA from polymerase chain reactions (PCR) and from sections of agarose gel using commercially available reagent kits. NAB Nanosep device performance was compared to the silica columns that come with commercial kits for either PCR cleanup or agarose gel extraction.

Materials and Methods

PCR product purification

A 1.7 kilobase PCR product was generated from a commercial plasmid using OneTaq◆ Quick-Load◆ 2X Master Mix from New England Biolabs. Multiple reactions were run and pooled together to ensure each spin device used was tested under identical loading conditions. The procedure to clean up the PCR product followed the manufacturer's protocol, with volumes adjusted to 500 µL for both the Pall and commercial kit spin devices as the NAB Nanosep device has a reservoir 500 µL capacity.

Agarose gel cleanup

A commercial plasmid was first linearized using restriction enzyme digestion. Each well of a 1% agarose gel was loaded with 0.6 µg of linear DNA. Following the gel separation step, bands from the gel were cut out and processed according to the manufacturer's protocol. Volumes were adjusted to 500 µL total as before.

Quantification of DNA

DNA quantification and purity were determined using a Qubit◆ fluorometer and NanoDrop◆ from Thermo Fisher Scientific, respectively. DNA purity was determined by $A_{260/280}$ and $A_{260/230}$ ratios. For $A_{260/280}$, ratios between 1.7-2.0 are considered pure, and for $A_{260/230}$, ratios between 2.0-2.2 are considered pure.

Results and Discussion

PCR product purification - Comparison of NAB Nanosep and silica column devices from a commercial PCR cleanup kit

Pall NAB Nanosep and commercial silica column spin devices were evaluated for PCR product recovery from a standard 50 µL volume reaction. Total DNA recovered along with the purity of the DNA was compared following cleanup. As indicated in Table 1, NAB Nanosep spin devices showed higher DNA recovery from this PCR product than commercial kit silica column spin devices. Both spin devices showed acceptable repeatability and purity. DNA quantification results obtained from analysis of the individual tubes tested are shown in Figure 1.

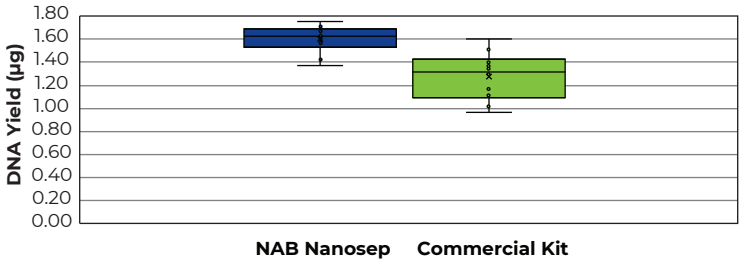
Table 1

DNA yield obtained after PCR product recover and cleanup using NAB Nanosep and commercial kit silica column spin devices. Data represents mean ± standard deviation (SD) of 10 spin devices from Pall and 10 spin devices from a commercial kit.

	DNA from PCR cleanup	
	NAB Nanosep	**Commercial column**
Quantity (µg)	1.60 ± 0.12	1.28 ± 0.21
$A_{260/280}$	1.76 ± 0.05	1.77 ± 0.02
$A_{260/230}$	2.22 ± 0.11	2.18 ± 0.09

Figure 1

DNA yield of samples used for PCR product recovery and cleanup. PCR products were cleaned-up with Pall NAB Nanosep and silica column spin filters using commercially available reagents for PCR product recovery. Data represents N=10 of both Pall NAB Nanosep and commercial kit devices.



Comparison of NAB Nanosep and silica column devices from a commercial agarose gel cleanup kit

Pall NAB Nanosep and commercial silica column devices were evaluated for agarose gel cleanup, recovery, and purity. Total DNA recovered along with the purity of the DNA was compared following cleanup. As indicated in Table 2, NAB Nanosep spin devices showed similar overall DNA recovery from an agarose gel slice as commercial kit silica column devices, with both showing high purity with respect to their $A_{260/280}$. Results obtained from analysis of the individual gel slices are shown in Figure 2.

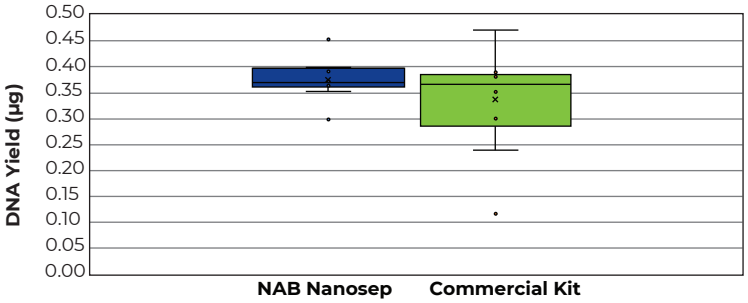
Table 2

DNA yield and purity obtained after agarose gel cleanup using NAB Nanosep and commercial kit silica column spin devices. Data represents mean ± SD of 10 spin devices from Pall and 10 spin devices from a commercial kit.

	DNA from agarose gel cleanup	
	NAB Nanosep	**Commercial column**
Quantity (µg)	0.38 ± 0.04	0.34 ± 1.0
$A_{260/280}$	1.81 ± 0.14	1.92 ± 0.12

Figure 2

Total DNA recovery of samples used for agarose gel cleanup. Individually collected gel slices were processed using a commercial kit with either Pall NAB Nanosep or silica column spin filters using commercially available reagents. Data represents N=10 of both Pall NAB Nanosep and commercial kit devices.



Summary

The aim of this study was to assess the use of NAB Nanosep centrifugal devices with commercially available reagents for PCR or agarose gel cleanup. It was evident from the experimental outcomes that Pall NAB Nanosep spin devices demonstrated robust performance when compared to the silica-based columns from commercially available kits. In both test conditions, Pall's NAB Nanosep showed more reproducibility and precision than the commercial kit silica columns by having smaller boxes with fewer points lying outside the standard deviations.

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